Unravelling microbial efflux through mathematical modelling

Jabbari, Sara

DOI:
10.1099/mic.0.001264

MICROBIOLOGY

REVIEW
Jabbari, *Microbiology* 2022;168:001264
DOI 10.1099/mic.0.001264

MICROBIOLOGY SOCIETY
OPEN ACCESS

Unravelling microbial efflux through mathematical modelling

Sara Jabbari*

Abstract

Mathematical modelling is a useful tool that is increasingly used in the life sciences to understand and predict the behaviour of biological systems. This review looks at how this interdisciplinary approach has advanced our understanding of microbial efflux, the process by which microbes expel harmful substances. The discussion is largely in the context of antimicrobial resistance, but applications in synthetic biology are also touched upon. The goal of this paper is to spark further fruitful collaborations between modellers and experimentalists in the efflux community and beyond.

DATA SUMMARY

No data were generated or reused in the preparation of this article.

INTRODUCTION

It is unquestionable that the rise in antimicrobial resistance, coupled with the drop in the discovery of new antibiotics, poses an enormous threat to human and animal health worldwide. While the search for new antibiotics is of course a priority, it is vital that all avenues to innovative treatments are also explored. This includes the development of antivirulence treatments that, rather than targeting the life cycle of the microbe, inhibit the ability of microbes to cause an infection. Much progress on these has been made in recent years, although in many cases this is currently limited to lowering (rather than clearing) the burden of the infection [1–3]. An alternative approach is to rejuvenate existing antimicrobials – rendering otherwise resistant infections susceptible to treatment by targeting the resistance mechanisms employed by the microbes [4].

Microbes use a multitude of antimicrobial resistance mechanisms [5], including (but not limited to) modifying or degrading the drug (for example, beta-lactamases [6]) or simply reducing the amount of drug that remains in the cell (for example, efflux pumps [7]). Coupled to these are detection and response mechanisms that facilitate the emergence of these transient behaviours [8]. This involves, for instance, complex nonlinear gene regulation networks and balancing single-cell and population-level survival, as well as having a range of alternative 'survival' mechanisms.

Understanding resistance (and how to inhibit it) therefore requires us to understand a multitude of factors, and how they affect each other. For instance, does downregulating one efflux pump simply lead to upregulation of an alternative one? [9] One tool that can aid with this and help to merge factors into a global picture is mathematical modelling. Translating the cellular interactions and processes into equations that can be probed either analytically (with pen and paper) or numerically (on a computer) vastly increases the scope of scenarios that can be considered and can open our eyes to hypotheses and predictions that may be difficult to access using a purely experimental approach that may be limited by time, resources and available technologies.

Using efflux pumps as an example, this review paper discusses some of the mathematical modelling work that has already enhanced our understanding of this important antimicrobial resistance mechanism. It is not intended to be a comprehensive or detailed review of all related models, rather the intention is that it is a useful resource to spark discussions and collaborations between experimentalists and theoreticians. The review is divided into three themes:

Received 12 August 2022; Accepted 14 October 2022; Published 21 November 2022
Author affiliations: [1]School of Mathematics and Institute of Microbiology and Infection, University of Birmingham, Birmingham, UK.
***Correspondence:** Sara Jabbari, s.jabbari@bham.ac.uk
Keywords: efflux pumps; interdisciplinary; mathematical modelling; multidisciplinary.
Abbreviations: ABC, ATP-binding cassette; BHI, brain heart infusion; LB, Luria-Bertani; MFS, major facilitator superfamily; MIC, minimum inhibitory concentration; PDR, pleiotropic drug resistance; RND, resistance nodulation division.

Impact Statement

This review paper aims to increase and enhance multidisciplinary collaborations within microbiology. In particular, it focuses on the role that mathematical modelling can play in understanding microbial efflux. Efflux pumps expel potentially harmful substances from within the cell and are a key antimicrobial resistance mechanism. Mathematical models can describe biological systems through equations that predict how interacting elements of the system evolve over time. In comparison to experimental work, they can be probed in a relatively straightforward fashion. For example, hundreds of different "experimental conditions" can be simulated in a click of a button. Furthermore, they can unravel the interacting elements to predict the important drivers of given system behaviours. By highlighting some of the impact that existing models have made on the efflux community to date, it is hoped that more researchers will initiate multidisciplinary collaborations both in the study of efflux and more widely in microbiology.

- Gene regulation networks;
- Single-cell versus population-level behaviour;
- Quantifying efflux.

We also comment on the limitations of modelling in the Discussion.

GENE REGULATION NETWORKS

A relatively large volume of mathematical modelling work has centred on understanding the architecture of the gene regulation networks that govern efflux regulation (for an accessible discussion of approaches to gene regulation network modelling, see [10]). The majority of these use differential equations; either ordinary – the solutions to which can be interpreted as average population behaviour – or stochastic, which allow for population heterogeneity. Much of cell survival is dictated by cells adapting to fluctuations in their environment on an appropriate timescale. Networks of genes, their mRNA and proteins (here, for example, those that constitute the efflux pump itself, or the regulators of efflux pump expression) have hence evolved within cells to detect signals (e.g. a stressor such as an antibiotic) and trigger downstream effects that render the cell more likely to survive in a given environment. These networks are often somewhat complex and the nonlinear interactions between components can make it difficult to predict the effect of overexpressing or deleting a component, for example. Alternatively, on a more fundamental level, it is often insightful to understand why a specific network has evolved in a particular way [11–13]. This can have implications for the design of circuits in synthetic biology. Mathematical modelling (where each component in the network is described by an equation that feeds into the other components' equations appropriately) readily lends itself to such investigations. For example, in creating switch-like responses [13, 14], in balancing multiple criteria to be optimized [15] (e.g. maximizing production of protein x whilst keeping energy expenditure below a threshold), or in understanding how to design circuits that interact with different species (e.g. via quorum sensing signalling [16]). Once a reliable model has been developed (and ideally tested against experimental data) it is straightforward (at least in comparison to equivalent efforts required to examine gene regulation networks experimentally) to alter expression of single or multiple genes, or to alter the structure of the network.

In the context of efflux pumps, the majority of mathematical models of gene regulation networks consider the *mar* operon that regulates production of the AcrAB-TolC efflux pump in *Escherichia coli* [17]: the *mar* operon (containing *marR* and *marA*) is subject to activation by MarA (which also activates the efflux pump genes) and repression by MarR. This repression is eased when stressors and MarR bind to each other, essentially resulting in the inactivation of MarR and allowing the positive feedback loop to dominate [18]. Much modelling work has been devoted to understanding why this combination of positive and negative feedback loops in *E. coli* may have evolved in this way. Work in [19], for instance, suggests that this architecture results in a fast and homogeneous (across a population of cells) response to a stressor, while in [20] it is demonstrated that the positive feedback amplifies MarA levels and the negative feedback could give rise to pulses in gene expression in the absence of a stressor (with consistently high levels of the protein in the presence of a stressor – consistent with [19]). However, in a later experimental study [21], the same group showed that the pulse-like behaviour could also be observed even without the negative feedback loop, illustrating that more is at play. It is frequently when models and data disagree that we learn the most. These pulses are also uncovered theoretically in [22] using a subtly different mathematical approach (Boolean modelling) where probabilities are assigned to the regulatory interactions of the gene regulation network. This approach and the transcriptional pulses are discussed further in the next section.

Naturally, gene regulation networks do not exist in isolation and additional inputs or outputs can be readily incorporated into models: [23] considers the additional transcription factors of Rob (a specific activator) and cAMP (a global activator), showing that the first may amplify the stress response, rendering the cell more robust to external changes in the second.

Meanwhile, [24] considers the downstream effects of MarA on diverse genes, finding that the response can vary dramatically by gene: some require extremely high levels of MarA for activation, while others may respond to lower levels but in a less homogeneous fashion across a population, for example.

With a view to identifying ways to disrupt efflux regulation for therapeutic gain, [25] incorporates multiple regulators of the *acrAB-tolC* system in *Salmonella*. Increasing the dimensionality of the model in this way (the more regulators there are, the more parameters there are that need to be estimated for the model) requires a similar increase in experimental data (in an ideal world, to parameterize a model you would have time series measurements of all the nodes in the system); [25] navigates this by exploiting mathematical techniques (nondimensionalization and asymptotic analysis) that enable predictions to be made based upon relative sizes of parameters, rather than absolute values. For example, we might be confident that transcription of gene x is roughly twice as fast as that of gene y without needing to know either individual rate. This approach is particularly useful in the absence of experimental data and allows the user to create more general hypotheses that do not rely on specific conditions, but it is technically challenging and can be understandably off-putting to researchers without a computational background.

One interesting input to regulation of *acrAB-tolC* is the Lon protease. Active degradation of proteins in bacteria is relatively rare since rapid cell division serves to dilute protein levels without the need for energy-intensive degradation; [26] seeks to understand, therefore, why such active degradation of MarA has evolved via the Lon protease, finding that the half-life of MarA impacts on how quickly downstream genes can respond and importantly (at least in the context of a stressor) how coordinated this response is. Such considerations will be vital if targeting degradation of an efflux activator to maximize internalization of an antibiotic.

Of course, the architecture of the *mar* operon is not unique to these genes and many of these models and predictions are applicable to a broader range of scenarios. Prajapat *et al.* [27] again focus on the *mar* circuitry but their findings are equally applicable to homologous systems. The authors explore how the dual activator/repressor system compares to hypothetical architectures with either only an activator (that upregulates itself and downstream genes in the presence of a stressor) or only a repressor (that represses itself and downstream genes in the absence of a stressor). By evolving the parameters governing the single-element architectures using a genetic algorithm, the authors try to match the graded response to a stressor that arises in the two element system. The activator-only system could only produce a switch-like response, and though the repressor system could display a graded response, surprisingly it did so at a higher cost to the cell in terms of protein production.

Control of the ATP-binding cassette (ABC) transporter genes in the yeast *Saccharomyces cerevisiae* via the pleiotropic drug resistance (PDR) transporters is examined in [28], again with a view to understanding the benefit to the cell of the particular motifs in the underlying gene regulatory architecture. This network comprises a feedforward loop (the activator upregulates a downstream target directly *and* via an intermediate regulator) and a positive feedback loop (the intermediate regulator is an autoinducer). The analysis suggests that, taken together, these accelerate the response time and amplify expression of the response gene. The positive feedback amplifies any noise within the system, potentially increasing heterogeneity across the population; something that has been posited as being beneficial for efflux as a transient resistance mechanism.

The wider overall feedback loop of efflux pumps – the pumps themselves reduce the concentration of their own (albeit indirect) inducer – is examined in the context of the design of synthetic gene circuits in [29]. It is demonstrated that the inclusion of a pump could dramatically alter the dose–response curves. Indeed, the importance of efflux pumps in biotechnology processes should not be overlooked. A key example is biofuel production: anything above wild-type production of biofuels could be toxic to the cells. Coupling increased biofuel production with increased efflux is a promising way to counter this and mathematical modelling could be instrumental in optimizing the process [30].

SINGLE-CELL VERSUS POPULATION BEHAVIOUR

The previously mentioned Boolean model in [22] takes a gene regulation network and converts it into a mathematical network (sometimes referred to as a graph) where each node represents a regulator or a downstream target and is either 'on' or 'off'. If an activator is on then it switches a downstream target on with an assigned probability. Conversely, if a repressor is on, the target is switched off with a given probability. One simulation involves introducing a stressor and seeing how the nodes respond over a number of time steps. The probabilistic nature of the regulations means each simulation will be different and can be interpreted as the potential behaviour of a single cell. Run thousands of times, the simulations can be analysed collectively to represent a population of cells. Note that a Boolean framework serves as a tractable example of creating an *in silico* population of cells but it is by no means the only method. For example, agent based modelling, in which the emergence of higher-level phenomena in a system is examined through the interactions (defined by a set of rules) of the lower-level systems and components ('agents'), can also distinguish population behaviour from that of single cells [31–33].

When aggregating the single-cell simulations in [22], the results show a homogeneous response (across the population) of efflux being switched on in the presence of a stressor (antibiotic). In the absence of stress, subpopulations of cells have efflux switched on, suggesting that a subset of the population are capable of responding quickly to stress. However, examining the single cell behaviour suggests that rather than it being a subpopulation that is predisposed to always having efflux genes ready to go, each individual cell may experience unsynchronized pulses of efflux activity, agreeing with [18, 19]. These pulses have been confirmed experimentally at the transcriptional level in a separate study [34] for a variety of different stress response genes in *E. coli*, including the *mar* operon. While modelling cannot provide definitive answers, it can open up avenues to explore the routes via which cells may have evolved to behave in this way.

Mixed populations of active and inactive effluxers have been explored in a number of studies (including some already discussed in the previous section). Wen *et al.* [31] use a combined experimental and modelling study to consider growth rates of the two subpopulations under antibiotic exposure, showing that mutants lacking efflux capabilities grow more slowly when surrounded by active effluxers than they do when surrounded by other efflux mutants. Their agent-based model predicts that the stronger the efflux and the more effluxers present, the more evident this impact will be. This supports an important connection between antibiotic resistance at the single-cell level and its potential impact at the population level or on co-cultures of mixed species: cells with resistance mechanisms can rapidly dominate a population in a stressed environment.

Effluxing comes at an energy cost and understanding how and when this trade-off becomes advantageous (or indeed stops being advantageous) is a question that can be explored through modelling. For example, by co-culturing cells with and without efflux pumps, [30] investigates how this trade-off is affected by the rate at which stress is introduced to the cells. The authors use a clever analogy with a leaking boat: if a leak is slow the water can be pumped out sufficiently efficiently that the boat stays afloat. In contrast, if the same quantity of water enters the boat in one go, the boat will likely sink. The study (that has strong quantitative agreement between model simulations and experimental data) finds that the benefit of expressing efflux pumps increases as the rate at which the stressor is added decreases. Importantly, the modelling work facilitates quantification of the amount and delivery time of an antibiotic required to optimize its therapeutic success. Inversely (since in this case enhanced efflux would be desirable), such calculations also have implications in synthetic engineering of bacterial biofuel production. This quantification of processes related to efflux is something that is naturally facilitated with the aid of mathematical modelling.

QUANTIFYING EFFLUX

Quantifying the efflux capabilities of a cell or a population of cells is more complex than it may at first seem. Since antibiotics ultimately kill cells, a surrogate substrate (e.g. ethidium bromide) is often used [35]. Cells typically employ multiple efflux pumps, so inhibition of one type of pump may not be sufficient to completely abolish efflux (see [36] for a modelling study that includes multiple RND, resistance nodulation division, pumps in *Salmonella*). Quantifying how much substrate is pumped out of a cell must also capture how much has first entered (or later re-entered) the cell, e.g. via a permeable membrane. Mathematical modelling can both draw all of these processes together and unravel them from each other. The framework in [37] is a neat example of this. The authors present a detailed combined modelling and experimental study whereby a model is developed that relates internalized antibiotic with growth rates of the cells, thus enabling them to make predictions about the efflux capabilities of bacteria (here via the MFS, major facilitator superfamily, effluxers in *E. coli*) given measurable data.

Similarly, [38] and [39] perform curve fitting to dose-dependent data to estimate the efflux capabilities of *E. coli* with respect to cephalosporins and penicillins, respectively, under specific conditions. The studies reinforce the distinction between minimum inhibitory concentrations (MICs) and efflux measurements: low MICs do not necessarily correspond with no efflux, as the study finds that the cells could expel substrates not previously believed to be effluxed because they could kill cells at relatively low concentrations (low MIC). This highlights the importance of permeability in overall internalized substrate concentration – the cells can only efflux what manages to get across the membrane in the first place.

This crucial balance between permeability and efflux is also investigated in [40, 41]. The first compares efflux efficiency for different types of pumps (e.g. a single pump versus two pumps in parallel or series across the cytoplasmic or outer membranes). The second fits two phases (initial substrate uptake followed by long-term adjustment) to previously published accumulation data [42]. The resulting parameters suggest early rapid accumulation of the substrate followed by a decrease in cell permeability. This compares favourably against more recent experimental data that demonstrate that changes in substrate accumulation in later growth are dominated by changes in permeability rather than efflux [43].

All modelling studies perform quantification in some sense (this may be absolute, relative or comparative) and those discussed in this section represent just a small sample of these.

DISCUSSION

Once a reliable mathematical model has been created, it is relatively straightforward to run *in silico* experiments – in some cases thousands of 'experimental conditions' can be 'tested' in a matter of minutes at the click of a button, and this need not always be performed by a specialist. These experiments can compare, for example, *in silico* wild-type strains against strains with single or multiple gene knockouts, 'rewiring' of the circuitry and different dosing strategies (this could be different quantities, different timings, tapered dosing, for instance). The possibilities are combinatorially vast. Further information can be gleaned by analytically examining the models – this can be more challenging technically, but can be particularly fruitful and less dependent on relevant experimental data. However, achieving the reliable model in the first place is a nontrivial exercise.

The modeller needs the appropriate level of knowledge of the biological system to determine which aspects of it need to be considered – too much detail and the model becomes intractable, not enough and it is too simplistic to yield significant insight beyond what is already known. While progress can be made without complementary experimental data, the two combined is significantly better and many of the studies discussed here demonstrate this. Sometimes, the most helpful data are not the data that would be routinely generated in the laboratory and so a certain level of open mindedness and trust is required (the same goes in the other direction, where, for example, modellers may be asked to answer questions that do not appear at first to have significant mathematical interest). This process is made easier if both complementary skills are housed within the same research group (or even better, researcher), but this is still somewhat rare. All of the above, however, can be achieved across researchers, departments and universities, provided that all parties communicate effectively.

Mathematical modelling is not infallible. Constructing a model where every possible factor is accounted for is unrealistic. Simplifying assumptions must be made and these must be continually tested. For instance, an assumption about the abundance of a particular protein (meaning its concentration need not be tracked in the model) may only be valid under certain conditions. If the model investigations stray into conditions where this assumption is invalidated, the model must be updated. Similarly, given how rare it is to have enough data to estimate rate parameters, it can be tempting to put too much confidence in those that have been estimated when the data used for this may have come from a limited set of experimental conditions. It is critical to still perform parameter sensitivity analyses to probe and question these estimates.

An often-heard criticism addressed at modelling is that, without experiments, it does not provide definitive answers (rather predictions and hypotheses), yet the same could be said of many *in vitro* experiments that are often performed under very specific conditions. Ethidium bromide as a surrogate for antibiotics has already been mentioned, and laboratory strains of bacteria (e.g. *E. coli* K12 or *Pseudomonas aeruginosa* PA01), and growth media (e.g. LB or BHI broth) are often chosen for experimental consistency over strains or solutions isolated from the environment or infections. These experimental 'simplifications' can themselves be considered to be models of a different kind.

Another criticism of modelling is that in some cases the model results simply lend more weight to what we already believe to know about a system, but the extra it can tell us (e.g. quantification of an aspect, predictions about the effects of perturbing the system) should not be overlooked. It is correct, however, that – whenever possible – models should not be considered in isolation. Often where they play their most useful role is in suggesting the optimal experimental avenue to answer a given question. Mathematical modelling can enhance and accelerate experimental work, not replace it.

While understanding efflux pumps is clearly important in combatting antimicrobial resistance, it is not the only thing that matters and mathematical models can be expanded to account for additional aspects: the bactericidal/bacteriostatic action of the antibiotic, interplay with the host immune response, dosing regimens and patient heterogeneity are just a few examples. In particular, mathematical modelling has a bright future in personalized medicine and the optimization of treatment regimes [44–46].

Mathematics is of course not the only discipline that can help – physics and chemistry are already routinely used in many laboratories, and collaborations with behavioural scientists will help to predict and counteract lack of adherence to proposed treatment regimes in personalized medicine, for example. There are countless ways in which multiple disciplines can come together to tackle problems in microbiology.

The goal of this paper was not to provide extensive details on how modelling has benefited the efflux community or vice versa (readers are strongly encouraged to read the original studies for many interesting findings), but rather to forge more multidisciplinary collaborations of the kind discussed here. When done effectively and with shared goals, bringing researchers together from different backgrounds helps to view problems from different perspectives, ask new questions and provide new routes to answers.

Funding Information
This work received no specific grant from any funding agency.

Acknowledgements
S.J. thanks Dr George Youlden and Dr Ryan Kerr for laying the groundwork for this article, as well as Dr Steven Murphy for his helpful scrutiny.

Author contributions
S.J prepared and wrote the article.

Conflicts of interest
The author declares that there are no conflicts of interest.

References

1. Theuretzbacher U, Piddock LJV. Non-traditional antibacterial therapeutic options and challenges. *Cell Host Microbe* 2019;26:61–72.
2. Hotinger JA, Morris ST, May AE. The case against antibiotics and for anti-virulence therapeutics. *Microorganisms* 2021;9:10.
3. Fleitas Martínez O, Cardoso MH, Ribeiro SM, Franco OL. Recent advances in anti-virulence therapeutic strategies with a focus on dismantling bacterial membrane microdomains, toxin neutralization, quorum-sensing interference and biofilm inhibition. *Front Cell Infect Microbiol* 2019;9:74.
4. Laws M, Shaaban A, Rahman KM. Antibiotic resistance breakers: current approaches and future directions. *FEMS Microbiol Rev* 2019;43:490–516.
5. Aslam B, Wang W, Arshad MI, Khurshid M, Muzammil S, *et al*. Antibiotic resistance: a rundown of a global crisis. *Infect Drug Resist* 2018;11:1645–1658.
6. Bush K. Past and present perspectives on β-lactamases. *Antimicrob Agents Chemother* 2018;62:10.
7. Du D, Wang-Kan X, Neuberger A, van Veen HW, Pos KM, *et al*. Multidrug efflux pumps: structure, function and regulation. *Nat Rev Microbiol* 2018;16:523–539.
8. Grkovic S, Brown MH, Skurray RA. Transcriptional regulation of multidrug efflux pumps in bacteria. *Semin Cell Dev Biol* 2001;12:225–237.
9. Blair JMA, Smith HE, Ricci V, Lawler AJ, Thompson LJ, *et al*. Expression of homologous RND efflux pump genes is dependent upon AcrB expression: implications for efflux and virulence inhibitor design. *J Antimicrob Chemother* 2015;70:424–431.
10. Karlebach G, Shamir R. Modelling and analysis of gene regulatory networks. *Nat Rev Mol Cell Biol* 2008;9:770–780.
11. Halfon MS. Perspectives on gene regulatory network evolution. *Trends Genet* 2017;33:436–447.
12. Janga SC, Collado-Vides J. Structure and evolution of gene regulatory networks in microbial genomes. *Res Microbiol* 2007;158:787–794.
13. Cherry JL, Adler FR. How to make a biological switch. *J Theor Biol* 2000;203:117–133.
14. Tiwari A, Ray JCJ, Narula J, Igoshin OA. Bistable responses in bacterial genetic networks: designs and dynamical consequences. *Math Biosci* 2011;231:76–89.
15. Otero-Muras I, Mannan AA, Banga JR, Oyarzún DA. Multiobjective optimization of gene circuits for metabolic engineering. *IFAC-PapersOnLine* 2019;52:13–16.
16. Balagaddé FK, Song H, Ozaki J, Collins CH, Barnet M, *et al*. A synthetic *Escherichia coli* predator-prey ecosystem. *Mol Syst Biol* 2008;4:187.
17. Piddock LJV. Clinically relevant chromosomally encoded multidrug resistance efflux pumps in bacteria. *Clin Microbiol Rev* 2006;19:382–402.
18. Alekshun MN, Levy SB. Regulation of chromosomally mediated multiple antibiotic resistance: the mar regulon. *Antimicrob Agents Chemother* 1997;41:2067–2075.
19. Rodrigo G, Bajic D, Elola I, Poyatos JF. Antagonistic autoregulation speeds up a homogeneous response in *Escherichia coli*. *Sci Rep* 2016;6:36196.
20. Garcia-Bernardo J, Dunlop MJ. Tunable stochastic pulsing in the *Escherichia coli* multiple antibiotic resistance network from interlinked positive and negative feedback loops. *PLoS Comput Biol* 2013;9:e1003229.
21. El Meouche I, Siu Y, Dunlop MJ. Stochastic expression of a multiple antibiotic resistance activator confers transient resistance in single cells. *Sci Rep* 2016;6:19538.
22. Kerr R, Jabbari S, Blair JMA, Johnston IG. Dynamic Boolean modelling reveals the influence of energy supply on bacterial efflux pump expression. *J R Soc Interface* 2022;19:20210771.
23. Rodrigo G, Bajić D, Elola I, Poyatos JF. Deconstructing a multiple antibiotic resistance regulation through the quantification of its input function. *NPJ Syst Biol Appl* 2017;3:30.
24. Rossi NA, Dunlop MJ. Customized regulation of diverse stress response genes by the multiple antibiotic resistance activator MarA. *PLoS Comput Biol* 2017;13:e1005310.
25. Youlden GH, Ricci V, Wang-Kan X, Piddock LJV, Jabbari S, *et al*. Time dependent asymptotic analysis of the gene regulatory network of the AcrAB-TolC efflux pump system in gram-negative bacteria. *J Math Biol* 2021;82:31.
26. Rossi NA, Mora T, Walczak AM, Dunlop MJ. Active degradation of mara controls coordination of its downstream targets. *PLoS Comput Biol* 2018;14:12.
27. Prajapat MK, Jain K, Saini S. Control of MarRAB Operon in *Escherichia coli* via autoactivation and autorepression. *Biophys J* 2015;109:1497–1508.
28. Charlebois DA, Balázsi G, Kærn M. Coherent feedforward transcriptional regulatory motifs enhance drug resistance. *Phys Rev E Stat Nonlin Soft Matter Phys* 2014;89:052708.
29. Diao J, Charlebois DA, Nevozhay D, Bódi Z, Pál C, *et al*. Efflux pump control alters synthetic gene circuit function. *ACS Synth Biol* 2016;5:619–631.
30. Langevin AM, Dunlop MJ. Stress introduction rate alters the benefit of AcrAB-TolC efflux pumps. *J Bacteriol* 2018;200:e00525-17.
31. Wen X, Langevin AM, Dunlop MJ. Antibiotic export by efflux pumps affects growth of neighboring bacteria. *Sci Rep* 2018;8:15120.
32. Gorochowski TE, Matyjaszkiewicz A, Todd T, Oak N, Kowalska K, *et al*. BSim: an agent-based tool for modeling bacterial populations in systems and synthetic biology. *PLoS One* 2012;7:e42790.
33. Lardon LA, Merkey BV, Martins S, Dötsch A, Picioreanu C, *et al*. iDynoMiCS: next-generation individual-based modelling of biofilms. *Environ Microbiol* 2011;13:2416–2434.
34. Sampaio NMV, Blassick CM, Andreani V, Lugagne JB, Dunlop MJ. Dynamic gene expression and growth underlie cell-to-cell heterogeneity in *Escherichia coli* stress response. *Proc Natl Acad Sci U S A* 2022;119:14.
35. Blair JMA, Piddock LJV. How to measure export via bacterial multidrug resistance efflux pumps. *mBio* 2016;7:e00840-16.
36. Youlden G, McNeil HE, Blair JMA, Jabbari S, King JR. Mathematical modelling highlights the potential for genetic manipulation as an adjuvant to counter efflux-mediated MDR in *Salmonella*. *Bull Math Biol* 2022;84:56.
37. Perez AM, Gomez MM, Kalvapalle P, O'Brien-Gilbert E, Bennett MR, *et al*. Using cellular fitness to map the structure and function of a major facilitator superfamily effluxer. *Mol Syst Biol* 2017;13:12.
38. Nagano K, Nikaido H. Kinetic behavior of the major multidrug efflux pump AcrB of *Escherichia coli*. *Proc Natl Acad Sci U S A* 2009;106:5854–5858.
39. Lim SP, Nikaido H. Kinetic parameters of efflux of penicillins by the multidrug efflux transporter AcrAB-TolC of *Escherichia coli*. *Antimicrob Agents Chemother* 2010;54:1800–1806.

40. Nichols WW. Modeling the kinetics of the permeation of antibacterial agents into growing bacteria and its interplay with efflux. *Antimicrob Agents Chemother* 2017;61:10.
41. Vesselinova N, Alexandrov BS, Wall ME. Dynamical model of drug accumulation in bacteria: sensitivity analysis and experimentally testable predictions. *PLoS One* 2016;11:e0165899.
42. Asuquo AE, Piddock LJ. Accumulation and killing kinetics of fifteen quinolones for *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *J Antimicrob Chemother* 1993;31:865–880.
43. Whittle EE, McNeil HE, Trampari E, Webber M, Overton TW, *et al*. Efflux impacts intracellular accumulation only in actively growing bacterial cells. *mBio* 2021;12:e0260821.
44. Colijn C, Jones N, Johnston IG, Yaliraki S, Barahona M. Toward precision healthcare: context and mathematical challenges. *Front Physiol* 2017;8:136.
45. Moore H. How to mathematically optimize drug regimens using optimal control. *J Pharmacokinet Pharmacodyn* 2018;45:127–137.
46. Hoyle A, Cairns D, Paterson I, McMillan S, Ochoa G, *et al*. Optimising efficacy of antibiotics against systemic infection by varying dosage quantities and times. *PLoS Comput Biol* 2020;16:e1008037.